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TITLE: Method for stably transforming plastids of multicellular plants

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INVENTOR-INFORMATION:

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CLAIMS:

What is claimed is:

1. A method for obtaining stably plastid-transformed cells of a multicellular plant, which comprises:

a) transforming plastids of said cells with a DNA molecule having:

i) a targeting segment comprising a DNA sequence substantially homologous to a pre-determined sequence of a plastid genome, said targeting segment enabling insertion of said DNA molecule into said plastid genome by homologous recombination with said pre-determined sequence;

ii) a selectable marker sequence disposed within said targeting segment, said selectable marker sequence being selected from the group consisting of: a sequence encoding a form of plastid 16S ribosomal that is resistant to spectinomycin or streptomycin, and a sequence encoding a heterologous protein that inactivates spectinomycin or streptomycin, said selectable marker sequence conferring a selectable phenotype to cells having substantially all plastids transformed with said DNA molecule; and

iii) at least one cloning site adapted for insertion of at least one additional DNA segment, said at least one cloning site being disposed within said targeting segment relative to said selectable marker sequence so as not to interfere with said conferring of said selectable phenotype;

b) maintaining said cells in a selection medium which permits survival of cells having transformed or non-transformed plastids, and which further permits expression of said selectable phenotype in cells having substantially all plastids transformed with said DNA molecule, said expression being indicative of stably plastid-transformed cells; and

c) selecting cells expressing said phenotype, thereby obtaining said stably plastid-transformed cells of said multicellular plant.

2. The method of claim 1, wherein the transformation comprises bombardment of the cells of the multicellular plant with microprojectiles coated with the DNA molecule.

3. The method of claim 1, wherein said selectable marker sequence encodes said plastid 16S ribosomal RNA.

4. The method of claim 1, wherein said selectable marker sequence encodes a heterologous protein that inactivates spectinomycin or streptomycin.

5. The method of claim 1 wherein the plastids are chloroplasts.

6. The method of claim 1 wherein the plant is Nicotiana tabacum.

7. The method of claim 1 wherein the selectable phenotype is green pigmentation.

8. A plant cell obtained by the method of claim 1.

9. The method of claim 1, wherein said DNA molecule further comprises a foreign DNA of interest as said at least one additional DNA segment.

10. The plant cell obtained by the method of claim 9 wherein the foreign DNA of interest encodes a protein.

11. A method for obtaining a stably plastid-transformed multicellular plant, which comprises:

a) transforming plastids of cells of said multicellular plant with a DNA molecule having:

i) a targeting segment comprising a DNA sequence substantially homologous to a pre-determined sequence of a plastid genome, said targeting segment enabling insertion of said DNA molecule into said plastid genome by homologous recombination with said pre-determined sequence;

ii) a selectable marker sequence disposed within said targeting segment, said selectable marker sequence being selected from the group consisting of: a sequence encoding a form of plastid 16S ribosomal RNA that is resistant to spectinomycin or streptomycin, and a sequence encoding a heterologous protein that inactivates spectinomycin or streptomycin, said selectable marker sequence conferring a selectable phenotype to cells having substantially all plastids transformed with said DNA molecule; and

iii) at least one cloning site adapted for insertion of at least one additional DNA segment, said at least one cloning site being disposed within said targeting segment relative to said selectable marker sequence so as not to interfere with said conferring of said selectable phenotype;

b) maintaining said cells in a selection medium which permits survival of cells having transformed or non-transformed plastids, and which further permits expression of said selectable phenotype in cells having substantially all plastids transformed with said DNA molecule, said expression being indicative of stably plastid-transformed cells; and

c) selecting cells expressing said phenotype, thereby obtaining said stably plastid-transformed cells of said multicellular plant; and

d) regenerating a plant from said stably plastid-transformed cells, thereby obtaining a stably plastid-transformed multicellular plant.

12. A multicellular plant obtained by the method of claim 11.

13. A multicellular plant the plastids of which have been stably transformed by a DNA molecule having:

a) a targeting segment comprising a DNA sequence substantially homologous to a pre-determined sequence of a plastid genome, said targeting segment enabling insertion of said DNA molecule into said plastid genome by homologous recombination with said pre-determined sequence;

b) a selectable marker sequence disposed within said targeting segment, said selectable marker sequence being selected from the group consisting of: a sequence encoding a form of plastid 16S ribosomal RNA that is resistant to spectinomycin or streptomycin, and a sequence encoding a heterologous protein that inactivates spectinomycin or streptomycin, said selectable marker sequence conferring a selectable phenotype to cells of said multicellular plant having substantially all plastids transformed with